

Using Charged Aerosol Detection as a Universal Approach to Analyze Pharmaceutical Salts Including Inorganic and Organic Counterions

Christopher Crafts¹, Marc Plante¹, Bruce Bailey¹, Paul Gamache¹, John Waraska¹, Ian Acworth¹, and Kannan Srinivasan²
¹ESA—A Dionex Company, Chelmsford, MA, USA; ²Dionex Corporation, Sunnyvale, CA, USA

ABSTRACT

Multiple analytical techniques are required to address the diverse nature of analytes (inorganic/organic, anionic/cationic, and differences in chromophores and hydrophobicity) used in pharmaceutical preparations. The objective of the study presented here was to develop the use of a more generic approach for the analysis of a broad range of analytes using HPLC with charged aerosol detection. A polymeric ZIC[®]-*p*HILIC HPLC column with a polar (zwitterionic) stationary phase was employed with a mobile phase of acetonitrile/methanol/IPA/water buffered with ammonium acetate. Here, the authors demonstrate the ability to rapidly analyze 13 commonly used inorganic, cationic, and anionic counterions, seven organic acids, and five organic bases simultaneously along with several active pharmaceutical ingredients (APIs) in < 25 min. Limits of detection were in the low ng (ppb) range with a linear response over three orders of magnitude and an intra-assay precision of less than 3% RSD. The recoveries of counterions calculated for six different, commercially available standards were shown to be within 5% of the theoretical results. The sensitivity of this approach can readily detect impurities at < 0.1% (w/w), while simultaneously measuring the API and counterion. As the approach uses LC-MS-compatible phases, the flow was split between the Charged Aerosol Detector (CAD[®]) and a single quad MS to generate mass/charge data for positive identification of APIs, organic counterions, and impurities. The method allows for the simultaneous measurement of cations and anions, as well as an API and its counterion(s), and ionic impurities on a platform-independent HPLC system without restrictions on organic content of the sample solvent.

INTRODUCTION

Inorganic cation and anion analyses are required for pharmaceutical formulations, product characterization, and environmental analyses. API salts influence their solubility, stability, and hygroscopicity so appropriate counterion choice is an important part of drug development. Ten of the most common pharmaceutical counterions are: chloride, bromide, nitrate, ammonium, sulfate, tosylate, phosphate, tartrate, ethylenediamine, and maleate.

Typically, ion chromatography (IC) with conductivity detection (ICCD) is employed, but individual IC techniques require dedicated, platform-dependent instruments for each suite of analytes. HILIC uses a polar stationary phase (e.g., zwitterionic) and a mobile phase that is highly organic but contains a small amount of aqueous/polar solvent. Inorganic cations and anions, organic acids and bases, as well as APIs can be separated using a polymeric zwitterionic column (ZIC) from Sequant with a binary gradient. Anions and cations in the same mixture can be measured simultaneously using the universal Corona[®] CAD detector. The LC system can also be configured with a postcolumn flow splitter to a MS detector for further characterization of the API.

Sensitivity and reproducibility are a requirement for the pharmaceutical industry. The wide dynamic range and the high sensitivity of CAD enables ionic impurities to be detected down to the 0.1% level. For each compound class, examples are shown for the calculation of experimental counterion concentration vs theoretical values.

METHOD CONDITIONS

Method Conditions	
Column	Sequant ZIC- <i>p</i> HILIC; 4.6 × 150 mm, 5 μm
Column Temperature	30 °C
Mobile Phase A:	15% 100 mM ammonium acetate pH = 4.68, 5% methanol, 20% IPA, 60% acetonitrile
Mobile Phase B:	50% 30 mM ammonium acetate pH = 4.68, 5% methanol, 20% IPA, 25% acetonitrile
Flow Rate	0.5 mL/min
Injection Volume	10 μL
Gradient	t = 0 min 20% B, t = 3 min 20% B, t = 24 min 70% B, t = 26 min 70% B, t = 32 min 15% B, t = 34 min 20% B, t = 40 min %B
Corona	100 pA range, no filter
Sample Vial	Polypropylene

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

INORGANIC IONS

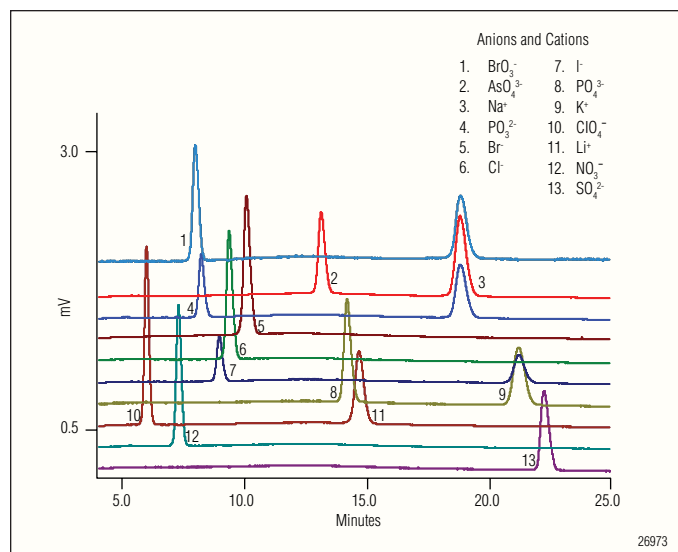


Figure 1. Overlays of 10 anions and three cations (10 μL injections of ~25 ppm salt solutions) analyzed using the gradient method.

ORGANIC ACIDS

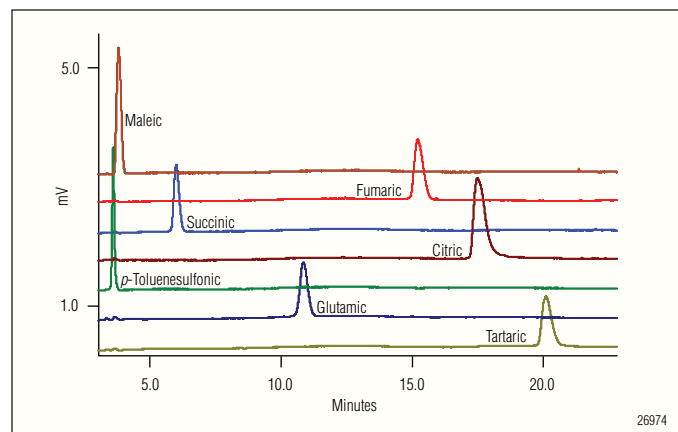


Figure 2. Overlays of seven organic acids (10 μL injections of ~60 ppm solutions) analyzed using gradient method.

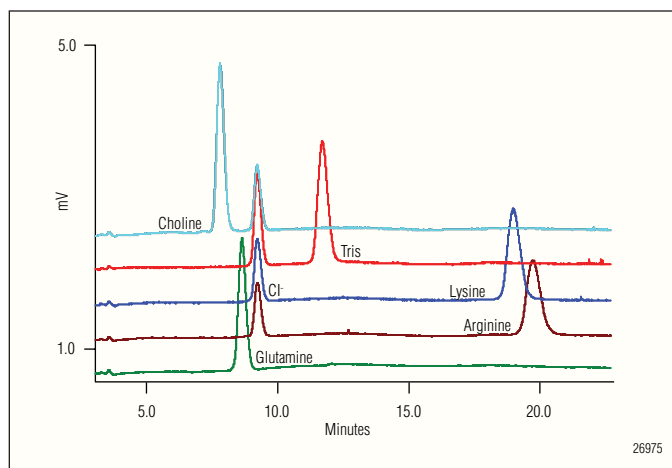


Figure 3. Overlays of five organic acids (10 μL injections of ~60 ppm solutions) analyzed using gradient method.

CAD-MS METHOD

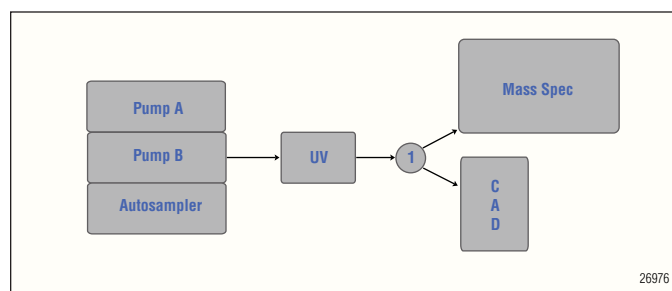


Figure 4. Schematic of the instrumentation setup used for the analysis. The 1 represents an adjustable flow splitter set to deliver 80 mL/min to the mass spectrometer and 0.42 mL/min to the Corona CAD when running the method at 0.5 mL/min total flow.

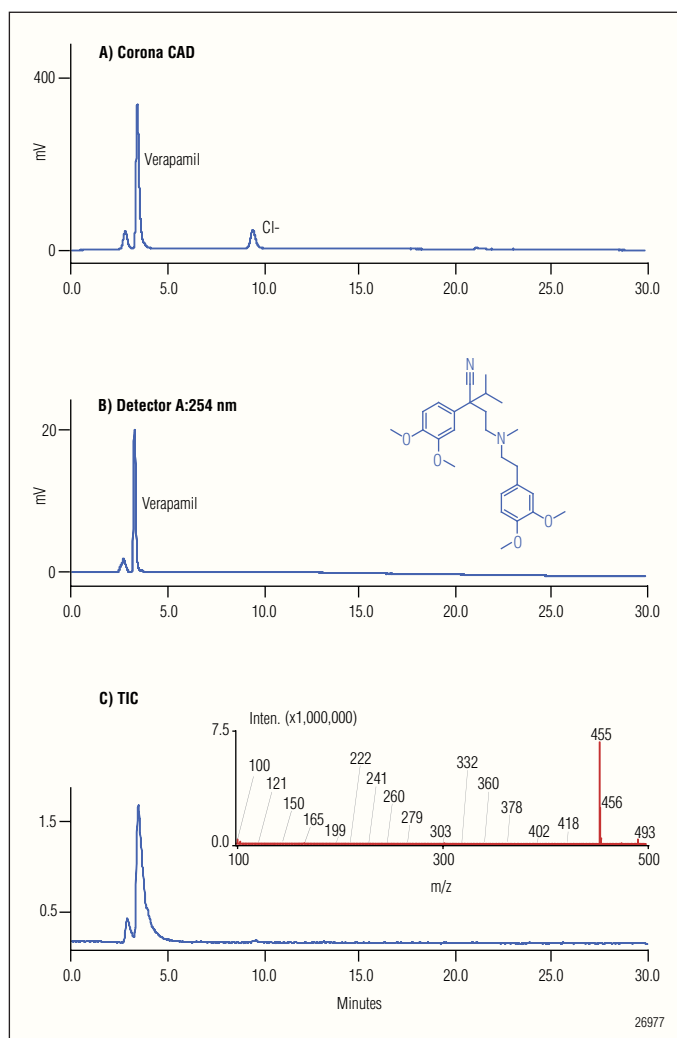


Figure 5. Verapamil hydrochloride at $\sim 1.2 \mu\text{g}$ on column (A) Corona CAD detection 100 pA full scale. (B) UV/vis detection at 254 nm with structure shown. (C) TIC mass spectrum scanning from 100–500 in positive ion mode. (Inlay) Mass spectrum for verapamil (MW = 455.6) retention time 3.6 min.

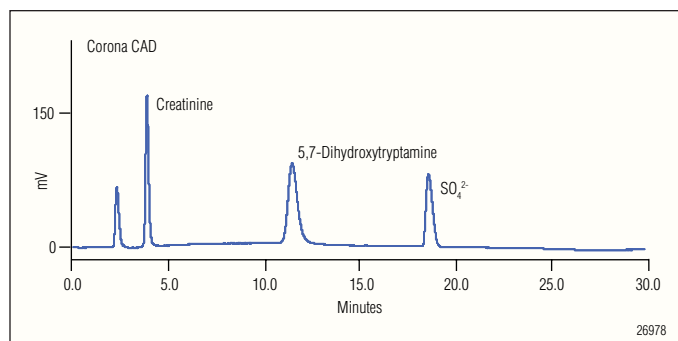


Figure 6. 5,7-Dihydroxytryptamine creatinine sulfate salt at $\sim 2 \mu\text{g}$ on column with Corona CAD detection 100 pA full scale.

ANALYSIS OF APIS AND COUNTERIONS

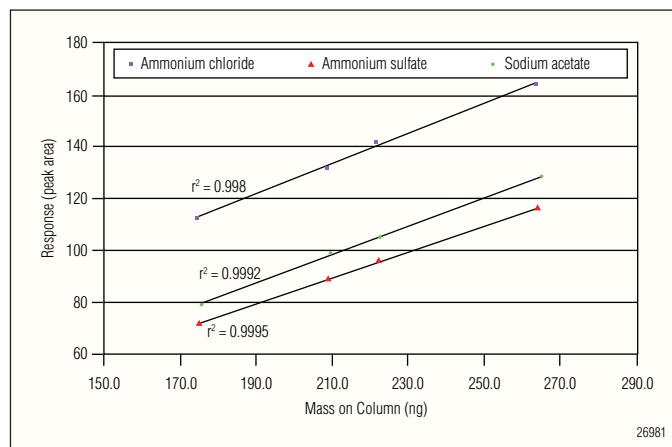


Figure 7. Target concentration curves for counterion analysis used for chloride, sulfate, and sodium.

Table 1: Counterion Comparison to Theoretical Values for APIs

Active Pharmaceutical Ingredient	Counter-ion	k' (relative retention)		Counterion %	
		API	Counterion	Theoretical	Experimental
Verapamil hydrochloride	Cl ⁻	0.27	2.4	7.2	7.0
Procainamide hydrochloride	Cl ⁻	1.3	2.4	13	12.7
Dextromethorphan hydrobromide	Br ⁻	N/D	2.8	21.3	22.4
Quinine sulfate dihydrate	SO ₄ ²⁻	0.86	7.0	12.3	11.9
Diclofenac sodium salt	Na ⁺	0.11	6.0	7.2	7.2
Enalapril maleate salt	C ₄ H ₃ O ₄ ²⁻	0.06	0.5	23.6	24.3

0.1% ION IMPURITIES

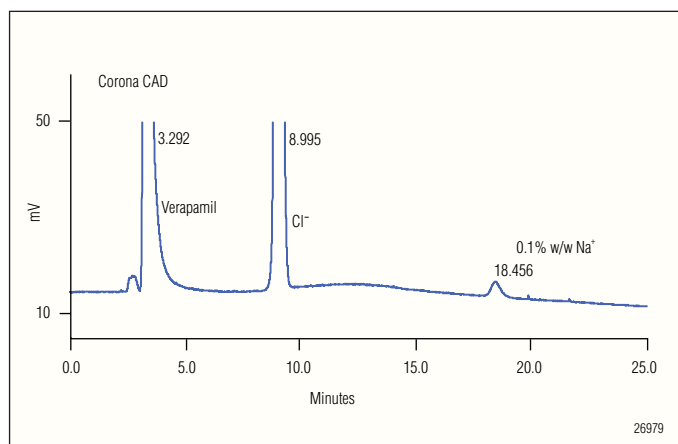


Figure 8. Chromatogram of a 10 μ L injection verapamil hydrochloride (0.7 mg/mL) in 80/20 acetonitrile/water with 0.1% by weight of sodium added.

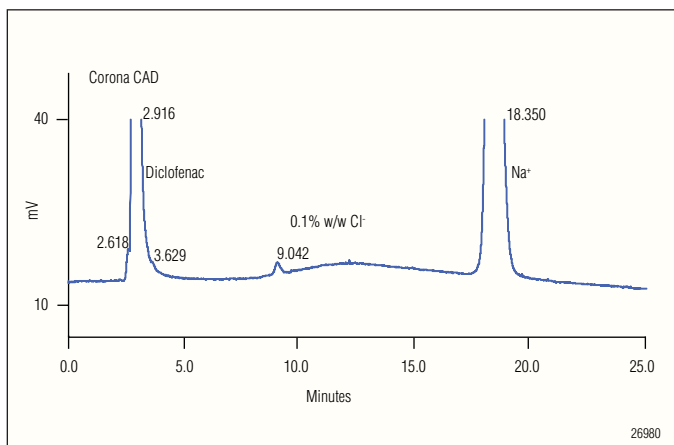


Figure 9. Chromatogram of a 10 μ L injection diclofenac sodium salt (0.3 mg/mL) in 80/20 acetonitrile/water with 0.1% by weight of chloride added.

DISCUSSION

As shown in Figure 1, a ZIC-*p*HILIC column and the CAD detector enabled the detection and partial resolution of 10 anions and three cations. The same single gradient system was able to resolve seven organic acids and five organic bases (presented in Figures 2 and 3, respectively). These data demonstrate the ability to measure eight of the top 10 most common counterions in a single method on a single HPLC system.

The HPLC-Corona CAD system can be used to measure a large range of organic compounds and ionic salts. However, it can also be used in conjunction with other detectors to provide an orthogonal technique to more fully qualify the composition of a sample. For example, the multidetector platform, presented in Figure 4, was used to characterize the composition of verapamil hydrochloride (Figure 5) and dihydroxytryptamine creatinine sulfate samples (Figure 6). In these examples, the Corona CAD detected all components in the sample, whereas the UV and the MS detected only the API. The MS data was used to better qualify the API. Such information may be used to assist the method development process.

The experimental percentages of the counterion for five APIs were calculated using calibration curves bracketed around the target concentration of the counterion. This allows a simple, linear regression ($r^2 \geq 0.998$ for all ions) to be used for the analysis of API counterions (Figure 7). The calculated experimental values for counterions correlated to within 5% of the theoretical values for all the components tested (see Table 1).

Regulatory authorities, including the ICH and USFDA, are placing greater emphasis on purity requirements and identification of all analytes and impurities contained in a formulation. This gradient HILIC-CAD approach is well suited for the measurement of low-level impurities. For example, Figures 8 and 9 show the measurement of low-level counterion impurities at the 0.1% w/w level.

CONCLUSION

- A single chromatographic method allows quantitative analysis of inorganic and organic counterions, APIs, and impurities at the 0.1% w/w level.
- The Corona CAD approach measures both anions and cations simultaneously on a single platform. It offers both time and cost savings when compared to conventional ICCD approaches.
- The Corona CAD method uses conditions compatible with LC-MS. An HPLC-CAD-UV-MS platform can be used to more fully characterize a sample.

REFERENCES

1. Haynes, D.A.; Jones, W.; Motherwell, W.D.S. Occurrence of Pharmaceutically Acceptable Anions and Cations in the Cambridge Structural Database. *J. Pharm. Sci.* **2005**, *94*, 2111–2120.
2. Risley, D.S.; Pack, B.W. Simultaneous Determination of Positive and Negative Counterions Using a Hydrophilic Interaction Chromatography Method. *LC-GC* **2006**, *24*, 776–785.

CAD and Corona are registered trademarks of Dionex Corporation. Third party trademarks herein are trademarks of their respective owners.

Passion. Power. Productivity.



Dionex Corporation

1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America

U.S./Canada (847) 295-7500

South America

Brazil (55) 11 3731 5140

Europe

Austria (43) 1 616 51 25 Benelux (31) 20 683 9768 (32) 3 353 4294
Denmark (45) 36 36 90 90 France (33) 1 39 30 01 10 Germany (49) 6126 991 0
Ireland (353) 1 644 0064 Italy (39) 02 51 62 1267 Sweden (46) 8 473 3380
Switzerland (41) 62 205 9966 United Kingdom (44) 1276 691722

Asia Pacific

Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 2764 2735
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190
Taiwan (886) 2 8751 6655

www.dionex.com



LPN 2611-01 10/10
©2010 Dionex Corporation